Development of a cutaneous mouse model of TSC and LAM by injection of human tuberin-deficient cells. Effects of chromatin remodelling agents

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Hamartomas are benign growths developed in tuberous sclerosis complex (TSC) patients in multiple organs. TSC is a genetically heterogeneous, autosomal dominant tumour suppressor gene syndrome linked to an altered *TSC1* or *TSC2* gene, encoding for hamartin and tuberin, respectively. A loss of both copies of *TSC2* is frequently observed in TSC-associated tumours such as renal angiomyolipomas but rarely in cortical tubers or skin lesions (Henske et al., 1996). The lack of tuberin results in mammalian target of rapamycin (mTOR) activation. Lymphangioleiomyomatosis (LAM) is a rare lung disease that may occur sporadically or associated to TSC. Approximately 90% of TSC patients develop skin hamartomas that can disfiguring or cause bleeding and pain but do not constitute life-threatening conditions. Current treatments are surgical and have the potential to leave scarring. Rapamycin, inhibitor of mTOR, has been tested without optimal results. The aim of this study is to develop a mouse model of TSC using tuberin-deficient cells with an epigenetic modification and to test the effect of the chromatin remodelling agent, 5-azacytidine. The available models of TSC do not completely reproduce the cutaneous features of the disease. *In vitro,* exposure to chromatin remodelling agents leads to tuberin expression in both cell types (Lesma et al, 2009; 2013). $3x10^6$ $TSC2^{-/meth}$ and LAM/TSC cells labelled with PKH 26 were subcutaneously administered in nude mice and were observed for 12 weeks and then sacrificed. Cell administration initially caused swelling near the site of injection that disappeared in few days without any tumor formation. After 2 weeks from the injection, skin thickness was observed in the back of the mice with the formation of hair such as observed *in vitro* (Li et al., 2011). Morphological evaluation indicated the presence of follicles in the thickened skin complete with sebaceous glands and hair shafts. Cells surrounding the hair bulb expressed phospho-S6, functional marker of TSC cells, and phospho-Erk. From the point of injection the cells migrated to the entire body of the mice in blood system and lymphatics. They were found in the blood (PCR) and in lymph nodes indicating an intrinsic tumorigenic potential. $TSC2^{-/meth}$ and LAM/TSC cells invade lungs causing enlargement of alveolar spaces and blood vessel formation. Both cell types reached the spleen, causing an increased number of megakaryocytes, and bone marrow, inducing myelofibrosis. Administration of 5-azacytidine (2mg/Kg/die) 4 times in a week reverted the skin alterations but caused a transient thickness of in lung tissue.

These data demonstrated that $TSC2^{-/meth}$ and LAM/TSC cells have migratory ability confirming the metastatic theory of cell dissemination in LAM and TSC. This animal model showed that cells-lacking tuberin appear to be the inciting cells for TSC skin alterations and could be important to study the morphogenesis of TSC skin lesions. The effect of 5-azacytidine on skin alteration provides insights into a new therapeutically approach for TSC in presence of an epigenetic modification.

Henske et al. (1996), *Am J Hum Genet*. 59: 400-6
Li et al. (2011), *Nat Commun*. 2, 235
Lesma et al. (2009), *Am J Pathol*. 174, 2150-9
Lesma et al. (2013), *J Pharmacol Exp Ther*. 345, 180-8